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दुग्ध एवं दुग्ध उत्पाद — नाईट्रेट एवं नाईट्राइट का अंश निर्धारण

भाग 1 कैडमियम अपचयन तथा स्पेक्ट्रोमेट्री पद्वति

(दूसरा पुनरीक्षण)

Indian Standard

MILK AND MILK PRODUCTS — DETERMINATION OF NITRATE AND NITRITE CONTENTS

PART 1 METHOD USING CADMIUM REDUCTION AND SPECTROMETRY

(Second Revision)

ICS 67.100.01

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

April 2008 **Price Group 7**

Dairy Products and Equipment Sectional Committee, FAD 19

NATIONAL FOREWORD

This Indian Standard (Part 1) (Second Revision) which is identical with ISO 14673-1 : 2004 'Milk and milk products — Determination of nitrate and nitrite contents: Part 1 Method using cadmium reduction and spectrometry' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Dairy Products and Equipment Sectional Committee and approval of the Food and Agriculture Division Council.

This standard was first published in 1981 based on the earlier version of International Standard, ISO 4099 : 1978 and subsequently revised in 1997 to align with the revised ISO 4099 : 1984 under dual numbering and its scope covered determination of nitrate and nitrite contents in cheese using cadmium reduction and spectrometry. Subsequently ISO 4099 was withdrawn and revised into three parts of ISO 14673 to include in its scope a variety of milk and milk products and also including two more methods for determination of nitrate and nitrite contents.

Subsequently to align with ISO 14673 on the subject, IS 9967 is now being revised into following three parts under the general title: 'Milk and milk products — Determination of nitrate and nitrite contents'

Part 1 Method using cadmium reduction and spectrometry and is identical to ISO 14673-1 : 2004;

Part 2 Method using segmented flow analysis (routine method) and is identical to ISO 14673-2 : 2004; and

Part 3 Method using cadmium reduction and flow injection analysis with in-line dialysis (routine method) and is identical to ISO 14673-3 : 2004.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards which are to be substituted in their places are given below along with their degree of equivalence for the editions indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 565 : 1990 Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings	IS 460 Specification for test sieves: (Part 1) : 1985 Wire cloth test sieves (*third revision*) (Part 2) : 1985 Perforated plate test sieves (*third revision*)	Technically Equivalent
ISO 648 : 1977 Laboratory glassware — One-mark pipettes	IS 1117 : 1975 Specification for one-mark pipettes (*first revision*)	do
ISO 835-1 : 1981 Laboratory glassware — Graduated pipettes — Part 1: General requirements	IS 4162 (Part 1) : 1985 Specification for graduated pipettes: Part 1 General requirements (*first revision*)	do

(*Continued on third cover*)

Indian Standard

MILK AND MILK PRODUCTS — DETERMINATION OF NITRATE AND NITRITE CONTENTS

PART 1 METHOD USING CADMIUM REDUCTION AND SPECTROMETRY

(Second Revision)

WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 14673|IDF 189 specifies a method for the determination of the nitrate and nitrite contents of milk and milk products by cadmium reduction and spectrometry. The method is applicable to

- whole and partly skimmed and skimmed dried milk;
- hard, semi-hard and soft cheeses;
- processed cheese;
- whey cheese, caseins and caseinates, and dried whey.

The method may be performed using automatic equipment, in particular by segmented flow analysis (SFA) or flow injection analysis (FIA), thus reducing cadmium contamination in laboratory work places and waste water

NOTE These methods are described in ISO 14673-2|IDF 189-2 and ISO 14673-3|IDF 189-3, respectively

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 565, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings*

ISO 648, *Laboratory glassware — One-mark pipettes*

ISO 835-1, *Laboratory glassware — Graduated pipettes — Part 1: General requirements*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
nitrate content
mass fraction of nitrate determined by the procedure specified in this part of ISO 14673 | IDF 189

NOTE The nitrate content is expressed as the mass in milligrams of nitrate ions (NO_3^-) per kilogram of product.

3.2
nitrite content
mass fraction of nitrite determined by the procedure specified in this part of ISO 14673 | IDF 189

NOTE The nitrite content is expressed as the mass in milligrams of nitrite ions (NO_2^-) per kilogram of product.

4 Principle

A test portion is dispersed in warm water, with precipitation of the fat and proteins, then filtration. The nitrate ions are reduced to nitrite ions in a portion of the filtrate by means of copperized cadmium.

A red colour is developed in portions of both unreduced filtrate and the reduced solution, by addition of sulfanilamide and *N*-1-naphthyl ethylenediamine dihydrochloride. Spectrometric measurements are carried out at a wavelength of 538 nm.

The nitrite content of the sample and the total nitrite content after reduction of nitrate ions are calculated by comparing the measured absorbances with those of a set of sodium nitrite calibration solutions. The nitrate content is calculated from the difference between these two contents.

5 Reagents

Use only reagents of recognized analytical grade unless otherwise specified.

5.1 Water, distilled or deionized, or water of equivalent purity, free from nitrate and nitrite ions.

To avoid the possible inclusion of small gas bubbles in the copperized cadmium column (9.1.6), freshly boil the distilled or deionized water and cool to room temperature. Use the thus-prepared water for the preparation of the column (9.1), to check the reducing capacity of the column (9.2), and to regenerate the column (9.3).

5.2 Cadmium granules, of diameter 0,3 mm to 0,8 mm.

Prepare cadmium granules, if not available commercially, as follows.

Place a suitable number of zinc rods in a beaker. Cover the rods with cadmium sulfate solution (5.3). Scrape the cadmium sponge from the rods from time to time over a period of 24 h. Remove the zinc rods and decant the liquid until only sufficient remains to cover the cadmium sponge. Wash the sponge two or three times with water. Transfer the cadmium sponge to a laboratory blender together with 400 ml of the hydrochloric acid working solution (5.7) and blend for a few seconds to obtain granules of the required size. Return the contents of the blender to the beaker and leave to stand for several hours, while stirring occasionally to remove bubbles. Decant most of the liquid and immediately copperize the granules as described in 9.1.

WARNING — Because of its toxicity, the used cadmium should be delivered as chemical waste to the relevant authorities.

5.3 Cadmium sulfate solution, $c(CdSO_4 8H_2O)$ = 40 g/l

Dissolve 40 g of cadmium sulfate solution in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.4 Copper(II) sulfate solution, $c(CuSO_4 \cdot 5H_2O)$ = 20 g/l

Dissolve 20 g of copper(II) sulfate in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.5 Hydrochloric acid (HCl), (ρ_{20} = 1,19 g/ml).

5.6 Dilute hydrochloric acid, $c(HCl) \approx 2$ mol/l.

Carefully add 160 ml of hydrochloric acid (5.5) to about 700 ml of water in a 1 000 ml volumetric flask (6.4) while regularly swirling the contents. Cool the contents to room temperature. Dilute to the mark with water and mix carefully.

5.7 Hydrochloric acid working solution, $c(HCl) \approx 0{,}1$ mol/l.

Add 50 ml of dilute hydrochloric acid (5.6) to a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.8 Zinc sulfate solution, $c(ZnSO_4 \cdot 7H_2O)$ = 535 g/l

Dissolve 53,5 g of zinc sulfate in water in a 100 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.9 Potassium hexacyanoferrate(II) solution, $c(K_4[Fe(CN)_6] \cdot 3H_2O)$ = 172 g/l

Dissolve 17,2 g of potassium hexacyanoferrate(II) trihydrate in water in a 100 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.10 Disodium ethylenediaminetetraacetate dihydrate (EDTA) solution ($Na_2C_{10}H_{14}N_2O_8 2H_2O$).

Dissolve 33,5 g of EDTA in about 900 ml water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.11 Solution I.

Carefully add 450 ml of hydrochloric acid (5.5) to a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.12 Solution II.

Dissolve by heating on a water bath (6.15) 0,5 g of sulfanilamide ($NH_2C_6H_4SO_2NH_2$) in a mixture of 75 ml of water and 5 ml of hydrochloric acid (5.5) in a 100 ml volumetric flask (6.4). Cool to room temperature. Dilute to the mark with water and mix. Filter the obtained solution, if necessary.

5.13 Solution III.

Dissolve 0,1 g of *N*-1-naphthyl ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) in water in a 100 ml volumetric flask (6.4). Dilute to the mark with water and mix. Filter the obtained solution, if necessary.

The solution may be stored for up to 1 week in a well-stoppered brown bottle in a refrigerator.

5.14 Sodium nitrite stock solution ($NaNO_2$).

Dry a few grams of sodium nitrite in an oven (6.16) at 110 °C to 120 °C to constant mass (i.e. until the difference between two successive weighings does not exceed 1 mg). Dissolve 0,150 g of the sodium nitrite in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.15 Sodium nitrite working solution.

Prepare the sodium nitrite working solution on the day of use. Transfer with a pipette (6.5), 10 ml of the stock solution (5.14) and 20 ml of the buffer solution (5.19) to a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix. The nitrite content of the sodium nitrite working solution is 1 µg/ml.

5.16 Potassium nitrate stock solution (KNO_3).

Dry a few grams of potassium nitrate in an oven (6.16) at 110 °C to 120 °C to constant mass (i.e. until the difference between two successive weighings does not exceed 1 mg). Dissolve 1,468 g of the potassium nitrate in water in a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix.

5.17 Potassium nitrate working solution.

Prepare the potassium nitrate working solution on the day of use. Transfer with a pipette (6.5), 5 ml of the potassium nitrate stock solution (5.16) and 20 ml of the buffer solution (5.19) to a 1 000 ml volumetric flask (6.4). Dilute to the mark with water and mix. The nitrate content of the potassium nitrate working solution is 4,50 µg/ml.

5.18 Ammonia solution (NH_3), (ρ_{20} = 0,91 g/ml).

If an ammonia solution of above-mentioned concentration is not available, an equivalent amount of a more concentrated ammonia solution may be used in 5.19 [e.g. 103 ml of a 35 % (mass fraction) ammonia solution (ρ_{20} = 1,19 g/ml)].

5.19 Buffer solution, pH 9,6 to 9,7.

Dilute 50 ml of hydrochloric acid (5.5) with 600 ml of water in a conical flask (6.3) and mix. Add 135 ml of the ammonia solution (5.18) and dilute to 1 000 ml with 215 ml of water and mix. Adjust the pH, if necessary, to between 9,6 and 9,7.

6 Apparatus

Clean all glassware thoroughly and rinse with distilled water to ensure that it is free from nitrate and nitrite ions.

Usual laboratory equipment and, in particular, the following.

6.1 **Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

6.2 **Sample container**, provided with an airtight lid.

6.3 **Conical flasks**, of capacity 250 ml, 500 ml and 1 000 ml.

6.4 **Volumetric flasks**, of nominal capacity 100 ml, 500 ml and 1 000 ml, complying with the requirements of ISO 1042, class B.

6.5 **Pipettes**, capable of delivering 2 ml, 4 ml, 5 ml, 6 ml, 8 ml, 10 ml, 12 ml, 20 ml and 25 ml, complying with the requirements of ISO 648, class A, or ISO 835-1. Where appropriate, burettes may be used instead of pipettes.

6.6 **Measuring cylinders**, of capacities 5 ml, 10 ml, 25 ml, 100 ml, 250 ml, 500 ml and 1 000 ml.

6.7 **Glass funnels**, of diameter 7 cm, with short stem.

6.8 **Filter paper**, medium grade, of diameter about 15 cm, free from nitrate and nitrite ions.

6.9 **Reduction column**, made of glass, an example of which is given in Figure 1.

6.10 Spectrometer, suitable for measuring absorbance at a wavelength of 538 nm, with cells of optical path length 1 cm to 2 cm.

6.11 Grinding device, appropriate for grinding the test sample, if necessary. To avoid loss of moisture, the device should not produce undue heat. A hammer shall not be used.

6.12 Laboratory mixer or **homogenizer**, with glass containers of capacity 250 ml or 400 ml, suitable for suspending test portions of cheese and whey cheese.

6.13 Test sieve, of woven wire cloth, of diameter 200 mm, nominal size of openings 500 µm, and a receiver complying with the requirements of ISO 565.

6.14 Magnetic stirrer.

6.15 Water bath, capable of boiling water.

6.16 Oven, capable of maintaining a temperature of between 110 °C and 120 °C.

7 Sampling

Sampling is not part of the method specified in this part of ISO 14673|IDF 189. A recommended sampling method is given in ISO 707.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Store the test sample in such a way that deterioration and change in composition are prevented.

8 Preparation of test sample

8.1 Dried milk and dried whey

Transfer the test sample to a sample container (6.2) of capacity about twice the volume of the test sample. Close the container immediately. Mix the test sample thoroughly by repeatedly shaking and inverting the container until a homogeneous sample is obtained.

8.2 Caseins and caseinates

8.2.1 Thoroughly mix the test sample, if necessary after transferring all of it to a sample container (6.2) of suitable capacity, by repeatedly shaking and inverting the container.

8.2.2 Transfer 50 g of the test sample to the test sieve (6.13). If the 50 g portion passes directly through the sieve, or almost completely, pass the whole mixed test sample (8.2.1) through the sieve.

8.2.3 If the test sample does not pass completely through the sieve, use the grinding device (6.11) to achieve that condition. Immediately transfer all the sieved test sample to the sample container (6.2) and mix thoroughly in the closed container. During these operations, take precautions to avoid any change in the water content of the product.

8.2.4 After the test sample has been prepared, proceed with the preparation of the test portion (9.4) as soon as possible.

8.3 Cheese

8.3.1 Prior to analysis, remove the rind or mouldy surface layer of the test sample so as to provide a test sample representative of the cheese as it is usually consumed.

8.3.2 Grind the test sample by means of an appropriate device (6.11). Mix the ground mass quickly and, if possible, grind a second time and again mix thoroughly. Clean the device after grinding of each sample. If the test sample cannot be ground, mix it thoroughly by intensive stirring and kneading.

8.3.3 As soon as possible after grinding, transfer the test sample to an airtight sample container (6.2) to await the determination which, preferably, should be carried out immediately. If a delay is unavoidable, take all precautions to ensure proper conservation of the test sample and to prevent moisture collecting on the inside surface of the container.

8.3.4 Do not examine ground cheese showing unwanted mould growth or beginning to deteriorate.

8.4 Whey cheese

Prepare the test sample as specified in 8.3.2.

9 Procedure

9.1 Preparation of the copperized cadmium column

9.1.1 Transfer an amount of cadmium granules (5.2) of 40 g to 60 g for each column to a 250 ml conical flask (6.3). Add sufficient hydrochloric acid working solution (5.7) to cover the cadmium. Swirl for a few minutes.

9.1.2 Decant the solution (9.1.1). Wash the cadmium in the conical flask with water until it is free from chloride ions (i.e. until reaction with silver nitrate is negative).

9.1.3 Copperize the cadmium granules by adding the copper(II) sulfate solution (5.4), of which about 2,5 ml of copper solution per gram of cadmium is needed. Swirl for 1 min.

9.1.4 Decant the solution (9.1.3) and wash the copperized cadmium immediately with water, taking care that the cadmium is continuously covered with water. Terminate the washing when the wash water is free from precipitated copper.

9.1.5 Fit a glass wool plug to the bottom of the glass column (6.9) intended to contain the copperized cadmium (see Figure 1). Fill the glass column with water.

9.1.6 Transfer the copperized cadmium to the glass column with minimum exposure to air. The height of the copperized cadmium shall be 15 cm to 20 cm. Avoid trapping air bubbles between the copperized cadmium granules. The level of the liquid shall not fall below the top of the copperized cadmium.

9.1.7 Condition the newly prepared column by running through it a mixture of 750 ml of water, 225 ml of the potassium nitrate working solution (5.17), 20 ml of the buffer solution (5.19) and 20 ml of the EDTA solution (5.10), at a flow rate not exceeding 6 ml/min. Wash the column with 50 ml of water.

9.2 Checking the reducing capacity of the column

9.2.1 Check the reducing capacity of the column at least twice a day, but also at the beginning and at the end of a series of determinations.

9.2.2 Pipette 20 ml of the potassium nitrate working solution (5.17) into the reservoir on top of the column. Immediately add 5 ml of the buffer solution (5.19) to the contents of the reservoir. Run the contents of the reservoir through the column at a flow rate not exceeding 6 ml/min. Collect the eluate in a 100 ml volumetric flask (6.4).

9.2.3 When the reservoir of the reduction column is nearly empty, wash the walls of the reservoir with about 15 ml of water. Repeat the washing with another 15 ml of water after the water has run off. When the second washing has run into the column, completely fill the reservoir with water. Run the complete contents of the reservoir through the column at maximum flow rate. Collect nearly 100 ml of the eluate.

9.2.4 Remove the 100 ml volumetric flask (9.2.3). Dilute its contents to the mark with water and mix well.

9.2.5 Pipette 10 ml of the eluate (9.2.4) into another 100 ml volumetric flask (6.4). Dilute the eluate to about 60 ml with water and mix. Proceed as specified in 9.7 to 9.9.

9.2.6 Calculate the percentage reducing capacity of the column (0,067 µg of NO_2^- per millilitre corresponds to 100 % reducing capacity) from the nitrite content obtained in 10.1 of the diluted eluate (9.2.5) and that determined from the calibration graph (9.9.4). If the reducing capacity is less than 95 %, regenerate the column as specified in 9.3.

9.3 Regeneration of the column

9.3.1 Regenerate the column at the end of each day of use or, if the check (9.2) indicates a loss of efficiency, more frequently.

9.3.2 Add about 5 ml of the EDTA solution (5.10) and 2 ml of hydrochloric acid working solution (5.7) to 100 ml of water and mix. Run the thus-obtained solution through the column at a flow rate of about 10 ml/min.

9.3.3 When the reservoir is empty, wash the column successively with water, with hydrochloric acid working solution (5.7) and again with water.

9.3.4 If the efficiency of the column still is not satisfactory, repeat the procedure specified in 9.1.7.

9.4 Preparation of test portion

9.4.1 Dried milk

Weigh, to the nearest 1 mg, approximately 10 g of the prepared test sample (8.1). Transfer the test portion quantitatively to a 500 ml conical flask (6.3). Add progressively to the test portion, 136 ml of water preheated to between 50 °C and 55 °C. Disperse the test portion by stirring with a glass rod or by shaking the conical flask.

9.4.2 Dried whey

Weigh, to the nearest 0,1 mg, approximately 5 g of the prepared test sample (8.1). Transfer the test portion quantitatively to a 500 ml conical flask (6.3). Add progressively to the test portion, 136 ml of water preheated to between 50 °C and 55 °C. Disperse the test portion by stirring with a glass rod or by shaking the conical flask. Cover the conical flask with aluminium foil or a watch glass and place it in the water bath (6.15) of boiling water for 15 min. Remove the flask from the water bath and wait until the temperature has dropped to between 50 °C and 60 °C.

9.4.3 Caseins

Weigh, to the nearest 1 mg, approximately 10 g of the prepared test sample (8.2). Transfer the test portion quantitatively to a 500 ml conical flask (6.3). Add progressively to the test portion, 136 ml of water preheated to between 50 °C and 55 °C and 10 ml of buffer solution (5.19). Disperse the test portion by stirring, using the magnetic stirrer (6.14).

9.4.4 Caseinates

Weigh, to the nearest 0,1 mg, approximately 2 g of the prepared test sample (8.2). Transfer the test portion quantitatively to a 500 ml conical flask (6.3). Add progressively to the test portion, 136 ml of water preheated to between 50 °C and 55 °C and 10 ml of buffer solution (5.19). Disperse the test portion by stirring, using the magnetic stirrer (6.14).

9.4.5 Cheese

Weigh, to the nearest 1 mg, approximately 10 g of the prepared test sample (8.3). Transfer the test portion quantitatively to the glass container of the laboratory mixer or homogenizer (6.12). Add progressively to the test portion, 144 ml of water preheated to between 50 °C and 55 °C. Mix in the mixer or homogenizer (6.12) until the test portion is well suspended.

9.4.6 Whey cheese

Weigh, to the nearest 1 mg, approximately 5 g of the prepared test sample (8.4). Transfer the test portion quantitatively to the glass container of the laboratory mixer or homogenizer (6.12). Add progressively to the test portion, 134 ml of water preheated to between 50 °C and 55 °C. Mix in the mixer or homogenizer (6.12) until the test portion is well suspended.

9.5 Extraction and deproteination

9.5.1 Dried milk, dried whey, caseins and caseinates, and whey cheese

9.5.1.1 Add to the test portion (9.4.1, 9.4.2, 9.4.3, 9.4.4 or 9.4.6 respectively), in the following order, 12 ml of zinc sulfate solution (5.8), 12 ml of potassium hexacyanoferrate(II) solution (5.9) and 40 ml of buffer solution (5.19), swirling thoroughly after each addition, and mix. In order to obtain a clear filtrate, leave the mixture in the conical flask for at least 15 min, but not longer than 1 h. Then pass through a filter paper (6.8), collecting the filtrate in a 250 ml conical flask (6.3).

9.5.1.2 It is essential to obtain a clear filtrate within the time specified. For this purpose, it may be necessary to use a larger volume of each precipitation reagent (5.8 and 5.9) (for example, if well-matured cheeses are analysed). Reduce the volume of the preheated water accordingly to maintain the volume of filtrate at about 200 ml.

NOTE The total volume of filtrate will be approximately 200 ml. This volume is assumed in the calculation (10.1 and 10.2)

9.5.2 Cheese

Add to the test portion (9.4.5), in the following order, 6 ml of zinc sulfate solution (5.8), 6 ml of potassium hexacyanoferrate(II) solution (5.9) and 40 ml of buffer solution (5.19), swirling thoroughly after each addition, and mix. In order to obtain a clear filtrate, leave the mixture in the glass container for at least 15 min, but not longer than 1 h. Then pass through a filter paper (6.8), collecting the filtrate in a 250 ml conical flask (6.3) (see 9.5.1.2 and note)

9.6 Reduction of nitrate to nitrite

9.6.1 Pipette 20 ml of the filtrate (9.5.1 or 9.5.2) into the reservoir on top of the prepared reduction column (9.1). Add 5 ml of buffer solution (5.19) to the contents of the reservoir. Mix by stirring with a small glass rod. Pass the contents of the reservoir through the column at a flow rate not exceeding 6 ml/min. Collect the eluate in a 100 ml volumetric flask (6.4).

9.6.2 When the reservoir of the reduction column is nearly empty, wash the walls of the reservoir with about 15 ml of water. After the water has run off, repeat the same washing with another 15 ml of water. After the second washing has run into the column, completely fill the reservoir with water. Pass the complete content of the reservoir through the column at maximum flow rate. Collect nearly 100 ml of the eluate in the 100 ml volumetric flask (6.4).

9.6.3 Remove the 100 ml volumetric flask. Dilute its contents to the mark with water and mix well.

9.7 Blank test

Carry out a blank test in parallel with the determination, but replacing the test portion in 9.8.1 by an equal volume of water.

9.8 Determination

9.8.1 Pipette equal volumes (for example 25 ml) of the filtrate obtained in 9.5.1 or 9.5.2 and of the eluate (9.6.3) into separate 100 ml volumetric flasks (6.4). Add water to each of the flasks to obtain a volume of about 60 ml and mix.

9.8.2 Add to the solution in the flask (9.8.1) 6 ml of solution I (5.11) and then 5 ml of solution II (5.12). Mix the solutions carefully and leave the flask protected from direct sunlight at room temperature for 5 min.

9.8.3 Add to the solution in the flask (9.8.2) 2 ml of solution III (5.13). Again mix these solutions carefully and leave the flask protected from direct sunlight at room temperature for 5 min. Dilute the contents of the flask to the 100 ml mark with water and mix.

9.8.4 Measure the absorbance of the solution obtained in 9.8.3 against that of the blank test (9.5) using the spectrometer (6.10) at a wavelength of 538 nm within 15 min.

9.9 Preparation of calibration graph

9.9.1 Pipette 0 ml (blank), 2 ml, 4 ml, 6 ml, 8 ml, 12 ml and 20 ml of the sodium nitrite working solution (5.15) into separate 100 ml volumetric flasks (6.4). Add water to each of the flasks to obtain volumes of about 60 ml.

9.9.2 Proceed as specified in 9.8.2 and 9.8.3.

9.9.3 Using the spectrometer (6.10), measure the absorbances of the sodium nitrite working solutions against that of the blank at a wavelength of 538 nm within 15 min.

9.9.4 Plot the absorbances obtained in 9.9.3 against the nitrite concentrations, in micrograms per millilitre, calculated from the amounts of sodium nitrite working solution added (9.9.1).

10 Calculation and expression of results

10.1 Nitrite content

10.1.1 Calculation of nitrite content

Calculate the nitrite content of the sample, ω_{N1}, using the following equation:

$$\omega_{N1} = \frac{20\,000\,c_1}{mV}$$

where

ω_{N1} is the nitrite content of the sample, in milligrams of nitrite ions per kilogram;

c_1 is the numerical value of the concentration read from the calibration graph, corresponding to the measured absorbance of the test solution (9.8.4), in micrograms of nitrite ions per millilitre;

m is the mass of the test portion (9.4), in grams;

V is the volume of the aliquot portion taken from the filtrate (9.8.1), in millilitres.

10.1.2 Expression of results

Express the results to 1 decimal place.

10.2 Nitrate content

10.2.1 Calculation of nitrate content

Calculate the nitrate content of the sample, ω_{N2}, using the following equation:

$$\omega_{N2} = 1{,}35\left[\left(\frac{100\,000\,c_2}{mV}\right) - \omega_{N1}\right]$$

where

ω_{N2} is the nitrate content of the sample, in milligrams of nitrate ions per kilogram;

c_2 is the numerical value of the concentration read from the calibration graph, corresponding to the measured absorbance of the test solution (9.9.4), in micrograms of nitrite ions per millilitre;

m is the mass of the test portion (9.4), in grams;

V is the volume of the aliquot portion taken from the eluate (9.8.1), in millilitres.

10.2.2 Calculation of nitrate content, considering the reducing capacity of the column

If the reducing capacity of the column is taken into account, calculate the nitrate content of the sample, ω_{N2}, using the following equation:

$$\omega_{N2} = 1{,}35\left[\left(\frac{100\,000\,c_2}{mV} \times \frac{100}{r}\right) - \omega_{N1}\right]$$

where r is the numerical value of the reducing capacity of the column at the end of a series of determinations.

10.2.3 Expression of results

Express the results to whole numbers.

11 Precision

11.1 General

The values for repeatability and reproducibility limits are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

NOTE The values for repeatability and reproducibility have been adopted from the former International Standards for the determination of nitrite and nitrate content in milk products, ISO 4099, ISO 6736, ISO 6739, ISO 6740 and ISO 8195. These International Standards were cancelled and replaced by ISO 14673-1:2001.

11.2 Repeatability

11.2.1 Nitrites

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 1 mg/kg of product.

11.2.2 Nitrates

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than

— for dried milk, dried whey, caseins, caseinates and cheese with a nitrate content < 30 mg/kg:	3 mg/kg,
— for dried milk, dried whey, caseins, caseinates and cheese with a nitrate content $\geqslant 30$ mg/kg:	10 % of the arithmetic mean of the results,
— for whey cheese with a nitrate content < 30 mg/kg:	5 mg/kg,
— for whey cheese with a nitrate content $\geqslant 30$ mg/kg	15 % of the arithmetic mean of the results

11.2.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than:

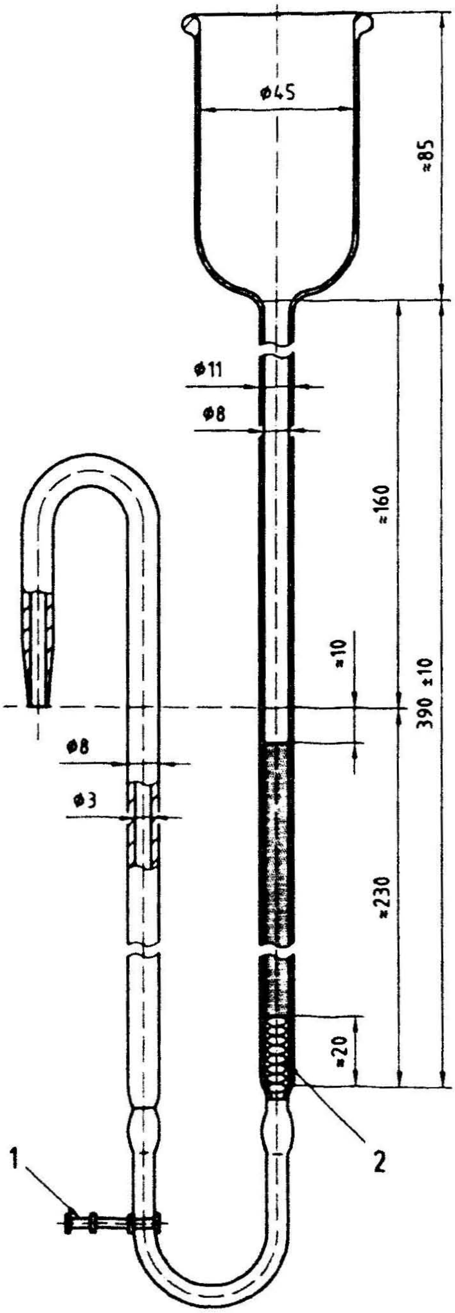
— for dried milk with a nitrate content < 30 mg/kg:	8 mg/kg,
— for dried milk with a nitrate content $\geqslant 30$ mg/kg:	25 % of the arithmetic mean of the results,
— for dried whey with a nitrate content < 30 mg/kg:	5 mg/kg;
— for dried whey with a nitrate content $\geqslant 30$ mg/kg	15 % of the arithmetic mean of the results
— for caseins and caseinates:	25 % of the arithmetic mean of the results,
— for cheese with a nitrate content < 30 mg/kg:	6 mg/kg,
— for cheese with a nitrate content $\geqslant 30$ mg/kg:	25 % of the arithmetic mean of the results
— for whey cheese with a nitrate content < 30 mg/kg	10 mg/kg,
— for whey cheese with a nitrate content $\geqslant 30$ mg/kg	15 % of the arithmetic mean of the results

12 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample,
- the sampling method used, if known,
- the test method used, with reference to this part of ISO 14673|IDF 189,
- all operating details not specified in this part of ISO 14673|IDF 189, or regarded as optional, together with details of any incidents which may have influenced the result(s),
- the test result(s) obtained or, if the repeatability has been checked, the final quoted results obtained.

Dimensions in millimetres



Key

1 screw clamp

2 glass wool plug

Figure 1 — Apparatus for nitrate reduction

Bibliography

[1] ISO 707:1997, *Milk and milk products — Guidance on sampling*

(Continued from second cover)

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 1042 : 1998 Laboratory glassware — One-mark volumetric flasks	IS 915 : 1975 Specification for one-mark volumetric flasks (*second revision*)	Technically Equivalent

In reporting the result of a test or analysis made in accordance with this standard, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

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